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# Integrated analysis of mechanistic considerations informative to cancer mode of action in the upper respiratory tract following formaldehyde inhalation

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## Abstract

### Purpose and Background:

Formaldehyde (FA) is used in both industrial applications and consumer goods, and exposure is associated with irritation of the nasal epithelium as well as pathologies such as hyperplasia, metaplasia and squamous cell carcinoma. This work presents a mode of action (MOA) analysis, conducted to examine the interdependence and collective contribution of these and other effects to upper respiratory tract (URT) carcinogenesis following inhalation exposure.

### Analytical Process and Initial Observations:

Available data were sorted by species and endpoint, and evaluated for consistency, magnitude, specificity, plausibility and coherence across multiple data-streams. DNA damage in the nasal passages is evident within days of exposure in animals, consistent with the clastogenicity observed in the nasal/buccal epithelium of humans after months to years of exposure. In conjunction with genotoxicity, FA elicits other effects in the respiratory epithelium, such as changes in cell proliferation rates, and cytotoxicity following ciliastasis and mucous flow interruption, which progresses to regenerative proliferation at higher concentrations.

### Evaluation of Coherence, and Assembly of Pathways:

As related events, genotoxicity, cytotoxicity and proliferation likely interact in a feed-forward manner, with each contributing to the amplification of initiated clones as a function of increasing FA exposure. For example, at low exposure levels both genotoxicity (DNA-protein crosslinks) and irritation may occur at the cellular level, triggering metaplastic transition and transient proliferation at the tissue level. This proliferative burst may indirectly augment direct formaldehyde-induced genotoxicity and trigger an outgrowth of nascent clones. Sustained regenerative proliferation may further enhance cellular genotoxicity and promote the expansion of mutant cells. By evaluating the potential for complementarity, this integrated analysis highlights the relationships and relative contributions of cytotoxicity, proliferation and genotoxicity to URT carcinogenesis.

## Temporal and Dose-Response Relationships Among Formaldehyde-Induced Effects in Rat Nasal Epithelium

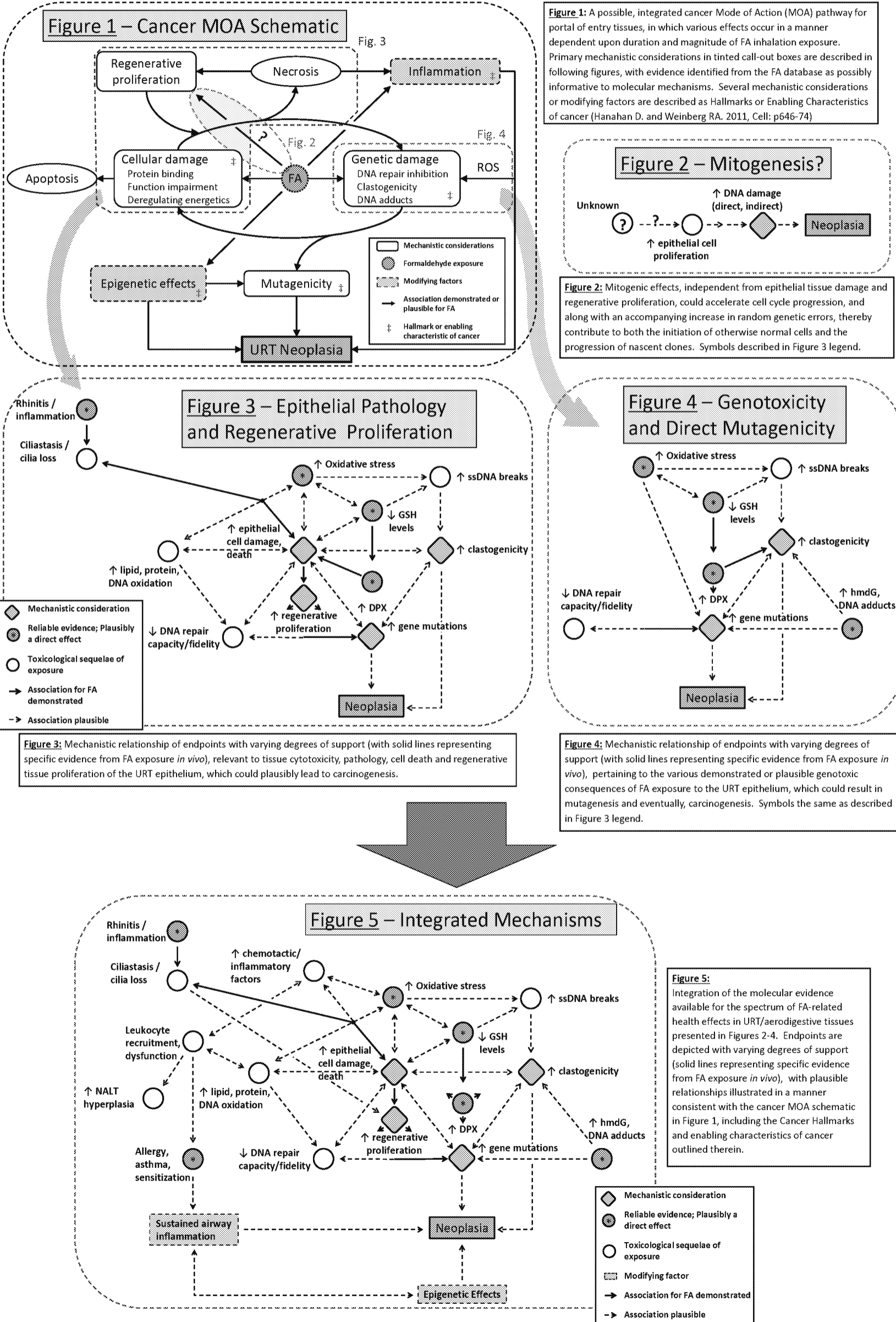
Duration of Exposure		Subchronic		Chronic		Subchronic		Chronic	
		Genotoxicity		Hyperplasia / metaplasia (Tissue pathology) <sup>a</sup>		DNA synthesis (Cellular proliferation) <sup>b</sup>		Tumorigenesis	
Exposure Level (mg/m <sup>3</sup> )	0-2	+	?	?	-	-/+	-/+	-	-/+
	2-7	++	?	?	+	+	++	+	++
	>7	+++	?	?	++	++	+++	++	+++
Exposure Level (mg/m <sup>3</sup> )	0-2	-/+ <sup>c</sup>	-	-	-	-	-	-	-
	2-7	++ <sup>c</sup>	+	+	-	-	-/+	-	-/+
	>7	+++ <sup>c</sup>	++	+	-	-	+++	-	+++

**Table 1**  
“—” indicates the absence of effects; “?” indicates apparent data gaps; “-/+” indicates an equivocal response, or evidence limited to the highest extreme of the exposure level range indicated; +, ++ and +++ and symbol size correspond to increasing magnitude or severity of an effect associated with formaldehyde exposure in rats.  
<sup>a</sup> Direct evaluation of tissue cytotoxicity (including cell death by apoptosis and/or necrosis) was not consistently reported, but for the purposes of this analysis was inferred by pathological reports of hyperplasia and/or metaplasia of the upper respiratory tract epithelium; as such, the associated compensatory proliferation may not be independent from genotoxicity-induced cell death and consequent tissue regeneration, mitogenic effects resulting from sterile inflammation or other sources.  
<sup>b</sup> Proliferation at the individual cell level was typically measured by incorporation of BrdU or [<sup>3</sup>H]-thymidine (i.e. DNA synthesis and/or repair), and was reported as an index normalizing affected (positive) cells to a designated portion of the total respiratory epithelium.  
<sup>c</sup> DNA synthesis has been evaluated following both continuous and intermittent exposures; while effects of continuous exposure are depicted herein for purposes of drawing comparisons across similar exposure scenarios, intermittent exposure may be also informative to some human experiences.

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## Data Integration: Mode of Action and Mechanistic Relationships



## Summary of *in vivo* Evidence for Formaldehyde-Associated Effects

Table 2: DNA Damage in the Aerodigestive/Upper Respiratory Tract			
Observations from the available database (references are illustrative, not exhaustive) <sup>a</sup>	Exposure (mg/m <sup>3</sup> ) <sup>b</sup>	Duration (days) <sup>c</sup>	
<i>Human (repeat occupational/environmental exposure)</i>			
↑ Micronucleus (MN) induction in nasal epithelium from workers (Ballarin et al., 1992; Ye et al., 2005)	≥ 0.1	≥ 365	
↑ MN induction in buccal epithelium from students, faculty, laboratory or factory workers (Titlenko-Holland et al., 1996; Viegas et al., 2010; Laderia et al., 2013)	≥ 0.2 / 0.06 – 0.6	90 / ≥ 365	
Limited ↑ MN in nasal epithelium from students; stronger association in centromere-negative MN (Titlenko-Holland et al., 1996; Suruda et al., 1993; Ying et al., 1997)	0.5 – 2	60 – 90	
<i>Human (acute controlled exposure)</i>			
NOE on MN incidence in nasal / buccal epithelial tissue (Zeller et al., 2011; Speit et al., 2007a)	≤ 1 / 17 (40 hrs)	5 / 10	
<i>Non-human primates (experimental)</i>			
↑ DNA-protein crosslinks (DPX) in the nasal mucosa and extranasal tissues of rhesus monkeys (Casanova et al., 1991)	≥ 0.9	3	
↑ DPX in the larynx/trachea/carina of rhesus monkeys (Casanova et al., 1991)	≥ 2	3	
↑ Exogenous FA <sup>14</sup> C/ <sup>3</sup> H-hmdG adducts in maxilloturbinate of cynomolgus monkeys (Moeller et al., 2011)	≥ 2	2	
↑ DPX in the lower respiratory tract (LRT) of rhesus monkeys (Casanova et al., 1991)	7	3	
<i>Rodents (experimental)</i>			
↑ DPX in the nasal epithelium of F344 rats (Casanova et al., 1994, 1989)	≥ 0.4	≥ 0.5	
↑ Exogenous FA <sup>14</sup> C/ <sup>3</sup> H-hmdG adducts in nasal epithelium of F344 rats (Lu et al., 2011; 2010)	≥ 0.9	≥ 1	
↑ Single strand DNA breaks (SSBs) in lung epithelial cells of Sprague-Dawley rats (Sul et al., 2007)	≥ 6	14	
NOE on DPX in bronchoalveolar lavage fluid or olfactory mucosa of F344 rats (Casanova-Schmitz and Heck, 1983; Neuss et al., 2010)	≥ 18	2	
NOE on MN incidence in nasal epithelium of F344 rats (Speit et al., 2011a; Neuss et al., 2010)	≤ 18	28	

**Associations with species, exposure duration and concentration**

- MN incidence in buccal epithelium is positively correlated ( $p < 0.01$ ) with exposure duration in human students or workers (Viegas et al., 2010); some association ( $p = 0.01 - 0.06$ ) with cumulative exposure (Titlenko-Holland et al., 1996; Suruda et al., 1993)
- N<sup>2</sup>-hmdG adduct levels positively associated with exposure concentration in maxilloturbinate of cynomolgus monkeys (Moeller et al., 2011), and associated with both concentration and duration in nasal epithelium of F344 rats (Lu et al., 2011; 2010)
- Single strand DNA breaks (SSBs) were positively associated with exposure level in lung epithelium from Sprague-Dawley rats (Sul et al., 2007)
- DPX incidence exhibited a decreasing concentration gradient with increasing anatomical distance from apical POE in both rhesus monkeys and F344 rats (Casanova et al., 1994b, 1991, 1989)

Table 4: Respiratory Tract Immune Dysfunction or Oxidative Damage			
<i>Associations with species, exposure duration and concentration</i>			
Positive associations with exposure concentration in human cohorts:			
○ <i>Infants:</i> Lower respiratory tract (LRT) infection frequency (Roda et al., 2011)			
○ <i>Children:</i> Allergic outcomes (Annesi-Maesano et al., 2012; Garrett et al., 1999)			
○ <i>Adults:</i> Nasal lavage ECP and lysozyme levels (Norback et al., 2000)			
Serum 15-F(2t) isoprostane levels in male workers were independently associated with 0.21 mg/m <sup>3</sup> formaldehyde exposure and smoking (Romanazzi et al., 2013)			
In the lungs of mice, GSH decreases were inversely associated with exposure concentrations, while ROS and MDA increases were positively associated with exposure concentrations (Ye et al., 2013)			

Table 3: Respiratory Tract Epithelial Pathology and Regenerative Proliferation			
Observations from the available database (references are illustrative, not exhaustive) <sup>a</sup>	Exposure (mg/m <sup>3</sup> ) <sup>b</sup>	Duration (days) <sup>c</sup>	
<i>Human (repeat occupational/residential exposure)</i>			
↓ Nasal patency (airway volume; Norback et al., 2000)	0.003 – 0.02	NR (repeat)	
↑ Rhinitis and nasal inflammation (Various)	0.05 – 1	NR (chronic)	
↑ General URT inflammation (Hanrahan et al., 1984; Liu et al., 1991)	≥ 0.2	NR (chronic)	
↓ Mucociliary function (Holmstrom and Wilhelmsson, 1988)	0.3	NR (chronic)	
↑ Hyperplasia, keratinization and metaplasia (Various)	0.05 – 0.6	NR (chronic)	
<i>Human (acute controlled exposure)</i>			
↑ Mucosal swelling (Falk et al., 1994)	0.07 – 0.2	< 1	
↑ Nasal and throat irritation (Various)	≥ 0.1	< 1	
↓ Mucociliary function (Andersen and Molhave, 1970)	0.3	< 1	
↑ Rhinitis and nasal inflammation (Various)	0.5	< 1	
<i>Non-human primates (experimental)</i>			
↑ Squamous metaplasia and hyperplasia in nasal turbinates of cynomolgus monkeys (Rusch et al., 1983)	≥ 1	180	
↑ Metaplasia, hyperplasia in nasal epithelium, nasopharynx and larynx of rhesus monkeys (Monticello et al., 1989)	7	≤ 42	
<i>Rodents (experimental)</i>			
↓ Mucociliary function, flow rate in F344 rats (Morgan et al., 1986a, c)	≥ 7	1 – 14	
↑ Nasal rhinitis and hyperplasia in F344 rats (Kamata et al., 1997; Appelmann et al., 1988; Woutersen et al., 1989)	≥ 2 / ≥ 12	≥ 365 / ≥ 90	
↑ Nasal squamous metaplasia in F344 rats (Kamata et al., 1997; Appelmann et al., 1988; Woutersen et al., 1989; Kerns et al., 1983b)	≥ 2 / ≥ 7	≥ 730 / ≥ 180	
↑ Nasal metaplasia in hamsters (Dalbey et al., 1982)	18	lifespan	

**Associations with species, exposure duration and concentration**

- Human URT irritation was positively associated with exposure concentrations from 0.6 – 3.7 mg/m<sup>3</sup> in controlled acute exposure trials (Kulle et al., 1987) as well as occupational studies (Holness and Nethercott, 1989; Horvath et al., 1988)
- Squamous metaplasia and hyperplasia in the nasal turbinates of cynomolgus monkeys was positively associated with chronic exposures ≤ 3.6 mg/m<sup>3</sup> (Rusch et al., 1983)
- Rat nasal metaplasia was increased at ≥ 2.5 mg/m<sup>3</sup> in a manner that was associated with exposure concentration and chronic duration (Kerns et al., 1983b)
  - Rat nasal mucociliary function and flow rate decreased at ≥ 7.4 mg/m<sup>3</sup> associated with exposure concentration and acute durations (Morgan et al., 1986a, c)

<sup>a</sup>No observed effect (NOE), treatment-associated increase (↑), treatment-associated decrease (↓)  
<sup>b</sup>The lowest effective concentrations (LECs) are presented where effects were associated with formaldehyde exposure, and the highest ineffective concentrations (HICs) in cases where NOE was reported  
<sup>c</sup>The shortest duration reported to elicit the specified effect is noted; “\*” indicates that positive associations were also reported at longer durations; “NR” indicates duration not specifically reported

Table 5: Direct Measurements of DNA Synthesis in the URT Epithelium			
<i>Associations with species, exposure duration and concentration</i>			
Epithelial cell proliferation increased in nasal and extra-nasal passage of rhesus monkeys exposed to 7.4 mg/m <sup>3</sup> for up to 42 days (Monticello et al., 1989)			
In anterior and posterior nasal passages of F344 rats, proliferation rates increased in a positive manner with increasing exposure (Andersen et al., 2010; Meng et al., 2010; Monticello et al., 1996, 1991), generally from:			
○ Subchronic exposure: 2.5 – 7.4 mg/m <sup>3</sup> (anterior); 2.5 – 12.3 mg/m <sup>3</sup> (posterior)			
○ Chronic exposure: 7.4 – 12.3 mg/m <sup>3</sup> (anterior); 7.4 – 18 mg/m <sup>3</sup> (posterior)			
○ Above the higher limit of these exposure levels, proliferation rates no longer increased in a concentration-dependent manner (i.e. apparent plateau)			
Magnitude of proliferation induced in F344 rats was positively associated with exposure durations up to 3 months (Andersen et al., 2010); proliferation rates were maximal around 1 – 3 months and progressively declined throughout 18 months of continuous exposure (Monticello et al., 1996)			

## Observations

- Genotoxicity:** Markers of direct genotoxicity, including DPX and hmdG adducts, correspond anatomically with and temporally precede subsequent URT neoplasia in experimental rodent models, consistent with increased MN levels induced in analogous nasal and buccal tissues and nasopharyngeal cancer in occupationally exposed humans.
- Tissue Damage and Regenerative Proliferation:** Increasing incidence or severity of similar nasal dysfunction and/or progressive tissue pathology is positively associated with exposure concentration or duration in humans and animal models, at earlier times and/or lower levels of exposure than those associated with carcinogenesis.
- Cellular Proliferation:** There is some evidence for cellular proliferation, as measured by nucleotide analog incorporation, at lower exposures and/or following shorter durations of exposure than those eliciting epithelial pathology (e.g. hyperplasia, metaplasia), suggesting that formaldehyde may induce proliferation through other mechanisms.
- Altered Immune Function and Oxidative Stress:** Nasal infection and/or allergic symptoms occur in humans following formaldehyde exposure, consistent with observations of epithelial tissue dysfunction, oxidative stress and sterile inflammation in rodent models, although only limited molecular data are available.